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Review

Cell division in the CNS: Protective response or lethal event in post-mitotic neurons?

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Abstract

Cell cycle events have been documented to be associated with several human neurodegenerative diseases. This review focuses on two diseases – Alzheimer's disease and ataxia telangiectasia – as well as their mouse models. Cell cycle studies have shown that ectopic expression of cell cycle markers is spatially and regional correlated well with neuronal cell death in both disease conditions. Further evidence of ectopic cell cycling is found in both human diseases and in its mouse models. These findings suggest that loss of cell cycle control represents a common pathological root of disease, which underlies the defects in the affected brain tissues in both human and mouse. Loss of cell cycle control is a unifying hypothesis for inducing neuronal death in CNS. In the disease models we have examined, cell cycle markers appear before the more well-recognized pathological changes and thus could serve as early stress markers—outcome measures for preclinical trials of potential disease therapies. As a marker these events could serve as a new criterion in human pathological diagnosis. The evidence to date is compatible with the requirement for a second "hit" for a neuron to progress cell cycle initiation and DNA replication to death. If this were true, any intervention of blocking 'second' processes might prevent or slow the neuronal cell death in the process of disease. What is not known is whether, in an adult neuron, the cell cycle event is part of the pathology or rather a desperate attempt of a neuron under stress to protect itself.

Keywords: Alzheimer; Ataxia Telangiectsia; Cell cycle; Neurodegeneration; APP mouse; *atm-deficient* mouse

1. Background

1.1. A normal biological cell cycle

The eukaryotic cell cycle, or cell-division cycle, is a series of events that lead to cell growth, DNA replication, chromosomal segregation and the creation of two daughters from an original mother cell. It is perhaps the most fundamental process of life. The cell cycle consists of four distinct phases: G1, S, G2 and M phases. G1 phase is a period of growth and preparation for division. Among the four phases it is the one with the most variability in length. During G1 a single cell grows to a point where it is ready to commit to division. G1 is followed by S phase in which is the DNA synthetic machinery replicates the genetic materials in the cell and chromosome number is doubled. The cell then prepares for division in a period known as G2 phase. Finally, during M phase, the chromosomes condense and move to opposite poles of the cell; the cytoplasm is split and two daughter cells are created. In cell cycle progression, two critical molecular classes—cyclins and cyclin-dependent kinases (CDKs) play key roles in the regulation of progression. The activation of these proteins varies with the phase of the cycle according to their own state of phosphorylation. In addition, a number of cell cycle inhibitors (such as the cyclin D inhibitors p16 and p27) act together to regulate a cell cycle and help to control the process by assessing damage and arresting progress at any of several defined checkpoints.

In the central nervous system (CNS), after young neuroblasts leave ventricular zone (VZ) or subventricular zone (SVZ), they become permanently post-mitotic cells. Once they become a mature neuron, the cell cycle is complete. The mature neuron will keep this status until the organism dies. The nature of this permanent exit from the cell cycle is still poorly understood. But

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the fact is that there are no known tumor that originates from a mature neuronal cell; the cancers that we refer to as "brain" tumors originate primarily from non-neuronal cells such as astrocytes, oligodendrocytes, and cells of the meninges. This is strong evidence that mature CNS neurons do not even try to divide after maturation. If they could, some neuron-originated tumor would have formed somewhere in some animal's brain. Although a small number of neurons destined for hippocampus and other regions are generated in the adult, their origin is a remnant of the subventricular zone rather than the result of a division of a truly postmitotic nerve cell.

1.2. Cell cycle associated cell death in post-mitotic neurons during development

The concepts of cell division and cell death seemed not only separate, but polar opposite. The former is a generative process that favors growth and development while the later is a destructive event that favors atrophy and loss. A substantial body of evidence now suggests, however, that the two processes are intimately related and use many similar mechanisms for their execution. In the past years, studies have shown the wide spread applicability of this linkage between cell cycle and cell death in mature neuronal cells.

Perhaps the first example of this was discovered by Feddersen et al. [1]. Using genetic engineering of transgenic mice, they showed that forcing a cell cycle in a mature neuron could lead to cell death. They took a powerful oncogene, the SV40 T antigen, and drove its expression in transgenic mice using a Purkinje cell-specific gene promoter, *pcp2*. The result was the induction of cell cycle related neuronal death rather than division. The functions of T-antigen include the binding of the endogenous cellular protein known as retinoblastoma (RB) [2]. RB is a nuclear protein that binds to a family of transcription factors known as the E2F family. If freed from RB, the action of E2F proteins is to drive the expression of a number of genes that leads to a cascade of events that initiates cell division and ensures its progression [3]. In normal quiescent cells, RB is inactivated through binding to E2F proteins and prevents the release of E2F family members to initiate the cell cycle, but the affinity of RB for E2F family members can be modulated through protein phosphorylation. Higher levels of RB phosphorylation lead to release of E2F, which initiates the cell cycle.

In their results, Fedderson et al. found that the transgenic founders that did indeed express T-antigen revealed an unexpected ataxic phenotype. The ataxia was caused by a large amount of cerebellar Purkinje cell death. In these transgenic mice, they observed that the Purkinje cells swelled in size and they could be labeled with the DNA precursor—bromodeoxyuridine (BrdU). But rather than dividing, the BrdU positive-labeled Purkinje cells died [1]. The suggestion was that the T-antigen oncogene had successfully initiated a cell cycle but for some unknown reasons the 'transformed' Purkinje cells could not complete the division.

This suggestion was also put forward to explain the phenotype of a different mutant mouse that was created in the following year. Three different labs reported an engineered null mutation in the mouse retinoblastoma gene [4–6]. All three found that, in the absence of RB, there were massive amounts of cell death in the developing embryonic CNS. Further, nerve cells failed to differentiate to an appropriately age-related stage in both CNS and PNS before they died. From these early findings, the concept has arisen that any forced cell division in a mature neuron is a lethal event.

A few years later, Herrup and Busser [7] extended this finding through the analysis of two cerebellar mutants where granule cell death occurs—*staggerer* (*Rora*^{sg}) and *lurcher* (*Grid*^{Lc}). Previous work had shown that granule cell loss was an indirect consequence of their failure to establish (*staggerer*) or maintain (*lurcher*) target contact. The direct destructive effects of the mutations, however, were on the granule cell target, the Purkinje cell. In both mutants, cerebellar granules cells rapidly die. Herrup and Busser showed that their deaths were accompanied by the re-expression of cell cycle proteins and incorporation of BrdU. These findings were evidence that, even in the natural setting of target-related cell death, post-mitotic neuronal cells that enter into an unscheduled cell cycle die.

Recently, the principal of cell cycling as a corollary of the cell death process has been extended further. Chen et al. [8] have shown that in mice lacking the cell cycle inhibitor, p19^{Ink4d}, there is a severe postnatal loss of hair cells. The death is cell cycle related as BrdU is incorporated into the normally post-mitotic cells.

Tissue culture model systems have also been developed and have provided some of the most detailed evidence establishing a linkage between cell cycle and cell death. Some of these data come from analyses of cell lines such as PC12 cells [9–12]. Using pharmacological approaches, Greene and colleagues have shown that drugs whose action blocks cell cycle advances are efficient at preventing the death of PC12 cells as well as sympathetic neurons [9–11,13]. Molecular genetic approaches have also been applied. Park et al. [11] used dominant negative forms of the Cdk4 and Cdk6 proteins to show that the cell death process induced by camptothecin are effectively blocked by following treatment with the G1/S blockers, such as deferoxamine, mimosine, as well as by the CDK inhibitors flavopiridol and olomoucine. The same authors have also shown that neurons treated with DNA damaging agents such as UV irradiation or camptothecin (a topoisomerase inhibitor) also require cyclin D and Cdk4/6 activity to induce neuronal death [14]. More detailed mechanistic data on the process was provided when it was shown that by blocking the cell cycle with cyclin-dependent kinase inhibition they could block neuronal cell death in culture [10,11,15]. These in vitro models are significant since many of them provide direct experimental evidence that, rather than merely being associated with cell death, an ectopic cell division is both necessary and sufficient to produce the death of neurons.

Taken together, these findings suggest that post-mitotic neurons are in a state of terminal differentiation and incapable of undergoing cell division. Nevertheless, they might retain certain elements of the cell cycle and have the capability of reactivating additional aspects of the replication mechanism when under

stresses. In another words, any events that force a mature neuron back into the cell cycle are lethal rather than mitogenic for the neuron.

2. Cell cycle events in neurodegenerative diseases and their mouse models

Over the past decades, there has been a growing body of evidence of cell cycle events associated with the progression of neuronal cell death. It seems only logical, therefore, to transfer this theme from developmental times in mutants or engineered lines of transgenic mouse to the field of the neuronal cell death in neurodegenerative diseases. We asked whether similar concepts and mechanisms apply to the occurrence of neuronal cell death in neurodegenerative diseases. And indeed, in recent years, several neurodegenerative diseases have been found associated with an apparent induction of a cell cycle in the brain areas where neurons are significantly lost in the disease conditions. Several laboratories have provided evidence for ectopic elevation and re-expression of cell cycle proteins in Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, stroke ataxia–telangiectasia and some forms of encephalitis [16–18]. In the current review, we will discuss the relationship of loss of cell cycle control and neuronal cell death in two neurodegenerative diseases that we have studied intensively—Alzheimer's disease [19–22] and ataxia telangiectasia [23]. Both diseases have well-established mouse models and we shall consider these as well.

2.1. The progression of Alzheimer's disease

Alzheimer's disease (AD) is the most common form of late-life dementia. It presents with a spectrum of significant clinical symptom that include deficits in the ability to form recent memories, executive dysfunction, impairment of cognition and other abnormal behavioral symptoms. Neuropathological findings in the AD brain are characterized by abnormal deposits of Aβ peptide and neurofibrillary tangles. The former consists of an abnormal cleavage product (Aβ) of a membrane protein known as APP; the latter consists of aggregates of hyperphosphorylated microtubule associated protein—tau. The density of neuronal cells (cell bodies and synapses) is significantly reduced in the frontal, entorhinal, hippocampal cortex and other limbic areas. In addition, certain sub-cortical neurons are involved in substantial amounts of neurodegeneration [24]. The loss of nerve cells in AD is regionally variable with severe losses observed in the hippocampus, frontal, entorhinal cortex, as well as in the basal nucleus of Meynert [25], the locus coeruleus [26,27] and the dorsal raphe [26,28].

There are two basic classifications of AD: familial AD (FAD) and late onset AD (LOAD). FAD has a relatively early age of onset, typically before the age of 65. It is relatively rare (5% of the total number of cases) and is nearly always caused by a single autosomal dominant mutation (PS1 and, more rarely, APP or PS2) with near 100% penetrance. It is a prominently studied form of Alzheimer's disease and the basis of most of the mouse modeling work. Despite this, 95% of the patients with AD suffer from the sporadic LOAD form of Alzheimer's, the biological basis of which is unknown. The onset of the LOAD is typically after the age of 65. As of this date, tremendous numbers of studies have been done with the goal of determining the biochemical pathways leading to the Aβ deposits and neurofibrillary tangles. For reviews of this topic the reader is referred to several recent references [29,30]. Yet the puzzle in the field has always been that despite the depth in which we understand the biochemical pathology (the Aβ plaques and the tangles of tau), the path that leads from these deposits to the regionally variable neuronal loss remains unknown.

Our laboratory has attempted to address this puzzle by focusing on the increasing evidence that the activation of cell cycle components is associated with cell death in the AD brain. We and others, have shown a close association between the neuronal cell death in AD and cellular processes that normally only occur during a mitotic cell cycle. Beginning with immunocytochemical evidence, many studies have reported that a wide range of cell cycle proteins, including cell cycle inhibitors, are elevated in neurons in at-risk regions of late stage of AD patients (Fig. 1A), but not in age-matched controls [19,31–39]. The cell cycle proteins whose levels have been reported to increase in AD include cyclin D, E [19,38,40], cdk4 ([19], PCNA [19,37], cyclin B[19], cdc2 [41–43] and Ki67 [37,38]). In addition, a number of studies have shown that several Cdk inhibitors are also present. These include p16 [3,32,44,45], p21 [45] and p105 [46]. Appearance of these cell cycle proteins is usually found only in actively mitotic cycling cells.

Yang et al. [21] have also reported that expression of cell cycle proteins is elevated in the at-risk areas during the early stages of neurodegeneration—a stage often referred to as mild cognition impairment (MCI). This is important as MCI is a term used to describe a subtle age-associated decline of human mental ability. Although there are controversies concerning the precise definition, a number of studies offer evidence that a high percentage of individuals diagnosed with MCI will progress to AD within 3–5 years [47]. Yang et al. [21] showed that in the brains of individuals who died with MCI the percentage of cell cycle positive neurons is nearly identical to that found in individuals who died with late stage AD. These findings suggest that neuronal death is related to cell cycle re-entry throughout the entire period of disease rather than a phenomenon that occurs only during the final stages preceding death.

A persistent and important question has been raised by these studies: does the immunocytochemical appearance of cell cycle proteins have any functional meaning in terms of actual cell cycle progression or is their appearance merely a reflection of a more global and non-specific dysregulation of protein synthesis? We have addressed this question by using fluorescent in situ hybridization (FISH) as a direct measure of DNA replication in the neurons of AD. Our lab [20] performed FISH using either large genomic probes to unique specific loci in the human genome or small probes to the highly repetitive DNA of the centromere of specific chromosomes. The FISH technique allows us to examine the numbers of copies of individual loci in neuronal nuclei. We found that, in AD brain, a significant fraction of the hippocampal pyramidal and basal forebrain

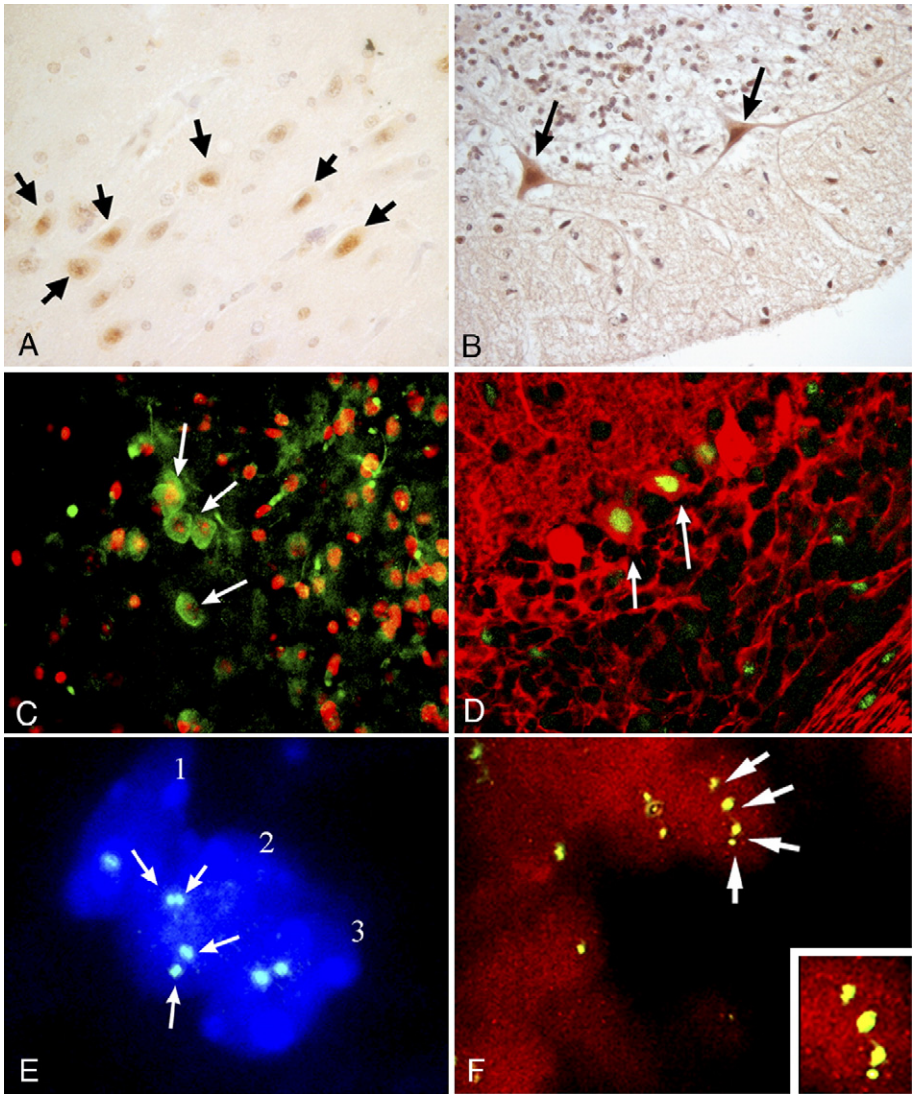

Fig. 1. Cell cycle events associated with degenerating neuronal cells in AD and A–T. (A) A hippocampal neuron from an AD patient specimen shows cyclin B positive immunostaining (from Yang et al. [21]). (B) PCNA expressed in the cerebellar Purkinje cells of diagnosed A–T patient. (C) In cortical neurons of the transgenic R1.40 mouse, the cell cycle marker, cyclin A, is re-expressed (arrows). (D) PCNA is expressed in the cerebellar Purkinje cells of *atm*-deficient mouse. (E) FISH analysis shows four bright hybridization signals from a unique locus in the mouse genome, indicating that DNA replication has occurred in the hippocampal neuron of transgenic R1.40 mouse (from Yang et al. [22]). (F) DNA replication also can be found in the Purkinje cells of the *atm*-deficient mouse (from Yang et al. [23]).

neurons have fully or partially replicated their DNA (2N to 4N) on separate genetic loci with different probes from three different individual chromosomes. This is a direct indication that DNA replication has occurred in the neuronal cells. No such anomalies were found in unaffected regions of the AD brain or in non-demented age-matched controls. These findings further prove that the ectopic expression of cell cycle proteins is sufficiently coordinated that a true cell cycle has been attempted and proceeded, most of the way through S-phase. Curiously, the cells are largely unable to progress further to complete their division. No cellular event of mitosis (M-phase) is found, not even the beginning of chromosome condensation. At the time this study was published, we proposed that cell cycling was initiated in at-risk neurons in AD and that the ultimate death of the nerve cells was attributable to the genetic imbalance caused by the nearly complete replication of the genome.

2.2. Mouse Models of Alzheimer's disease

Many different transgenic mouse models have been generated that recreate the genetic changes in found in familial AD [48–52]. Transgenic mice expressing mutant human APP genes often exhibit an age-related development of diffuse and neuritic plaques, with plaque severity burdens equivalent to those found in advanced cases of AD. These APP transgenic mouse models have been used and proven to be a valuable resource in the exploration and design of disease therapies [53]. In addition, the AD mice show microglial activation, astrocytosis, and changes in neuronal cytoskeletal proteins including tau [50,54,55]. Many of these models have also been shown to have significant memory deficits [49–51]. Despite these findings and their similarities to the human disease conditions, none of the mouse models has yet been shown to develop the typical neurofibrillary

tangles and in only a few has any significant loss of neuronal cell bodies been reported (for review see reference [56]). Recently the LeFerla lab has been able to create a more complete pathological picture by incorporating three transgenes into a single mouse [51,57]. These animals have provided valuable insights, but the fact remains that they are not true models of AD as their genetics is complex and not representative of even the more rare forms of FAD.

It has been a curious observation that in virtually all AD mouse models, even those with a close genetic mimicry of FAD, the loss of neurons is either non-existent or relatively mild. The reason for the discrepancy between the human and mouse neurodegenerative phenotype is unclear. As cited above, there are a large and growing number of conditions in mouse in which neuronal cell death is associated with re-entrance into a lethal cell cycle. These include the *retinoblastoma* deficient mouse [4–6], cerebellar target-related cell death [58], oncogene expression in maturing neurons [1], oxidative stress as suggested by the *harlequin* mouse [59], as well as MCA occlusion models of stroke [17] and the SOD-1 mouse model of amyotrophic lateral sclerosis [60,61]. These *in vivo* studies, combined with elegant studies in cell culture [62,63] strongly implicate the initiation of cell cycling as a causative factor in the death of these neurons.

Because of this close association between the aberrant neuronal cell cycle and neuronal degeneration in several neurodegenerative disorders, it occurred to us to ask the reasonable question of whether the cell cycle phenotype was preserved in the neurons of the AD mouse model, even in the absence of overt neuronal degeneration. This indeed proved to be the case. Yang et al. reported that abnormal cell cycle processes have begun in the appropriate neurons in three different transgenic models of AD—R1.40, Tg2576 (or Tg2576/PS1) and APP23 [22]. Both the ectopic expression of cell cycle proteins (Fig. 1C) and the occurrence of the true DNA replication (Fig. 1E) have been documented in these three transgenic lines. Thus these normally post-mitotic neuronal cells have attempted a cell cycle process that strongly resembles a mitotic cell division. With a more detailed study of when the initiation of cell cycle events occurs in the transgenic R1.40 line, we have shown cell cycle alterations are evident at 6 months of age. This is well before the first Aβ deposits that are not found in these animals until after the mouse is 1 yr old. Furthermore, the neuronal cell cycle activity precedes the appearance of $CD45^+$ activated microglia, and it occurs in an anatomical pattern that recapitulates the selective neuronal vulnerability observed in AD. This suggests that the ectopic initiation of cell cycle processes in neurons is an early sign of neuronal distress in both human and mouse AD. The relative timing of cell cycle appearance in the R1.40 mouse suggests that neither the activated microglia nor the amyloid plaques themselves are necessary to initiate the pathogenic events in AD. The authors reported also that the expression level of cell cycle proteins vary among the different cortical regions of the transgenic brain. In the sub-cortical structures, cell cycle proteins are re-expressed in the neurons of the dorsal raphe and the locus coeruleus, but not in the substantial nigra, which was considered not involved in pathologic changes in AD. This corresponds well with findings in Alzheimer's disease. Curiously, the cholinergic neurons in the mouse basal forebrain are cell-cycle negative, yet the neuronal loss in this cell group is severe in AD. It is remarkable that the CCEs in mouse are correct in their anatomical appearance relative to the human disease. The markers appear most strongly in the hippocampal formation (including entorhinal cortex) and in frontal cortical regions. Further, just as in the human disease the adrenergic neurons of the locus coeruleus and the serotonergic neurons of the dorsal raphe are also affected by the abnormalities in cell cycle regulation.

The study illustrates that cell cycle events are correlated with neuronal death in three individual different mouse models of human Alzheimer's disease just as in the human condition itself. We must face the fact, however, that none of the three transgenic mouse lines has any significant loss of neuronal cells. This raises an unexpected conclusion: the cell cycle events may be a necessary first step in the neuronal cell death process in AD, but they are not sufficient induce cell death. This means the initiation of the cell cycle, including DNA replication, can be followed by many months during which the aneuploid neurons persist in what we can assume is a near functional state. This is implied by the fact that the R1.40 animals have mild behavioral abnormalities [56]. This suggests that the cell cycle induced neuronal cell neurodegeneration is a slow prosecution of a complex cell death program. In retrospect this is entirely consistent with the calculations of Busser et al. [19] and Yang et al. [20] in their studies of human Alzheimer disease brains.

2.3. Human Ataxia Telangiectasia

Ataxia telangiectasia (A–T) is an autosomal recessive disorder characterized by cerebellar ataxia, progressing to substantial loss of motor function. On pathological examination cerebellar Purkinje and granule cell degeneration is noted [64] as well as neuronal loss in striatum and substantia nigra at the more advanced stages of the disease [65–68]. Other observed phenotypes in this complex disorder include retinal telangiectasia, immunodeficiency, radiosensitivity, infertility, predisposition to malignancies and progressive neuronal degeneration [64,69,70]. In A–T patients, the extent of the Purkinje cell loss is correlated with the severity of the clinical phenotype.

A disease gene has been identified, Ataxia–telangiectasia mutated (ATM). The protein encoded by this gene is a 370 kDa serine/threonine kinase. The primary sequence shows analogy with the catalytic subunit of phosphatidyl-inositol-3-kinase (PI3K) [71–75]. Many of the substrates of ATM are known to be involved in DNA double strand break detection and cell cycle arrest [76–80]. ATM is a central player in an elaborate network of proteins that responds to the presence of DNA double strand breaks and, in the aggregate, arrest the cell cycle until the damage to the genome can be repaired. Because the full A–T syndrome includes defects in cell cycle regulation plus neuronal degeneration, we were interested in determining whether the loss of neurons was in any way related to the cell cycle control defects.

The cell cycle checkpoint failure caused by the dysfunction of ATM gene in A–T has been extensively studied from the standpoints of the immune deficiency and tumor susceptibility. Evidence from skin fibroblasts, myeloid cells and other cells cultured from A–T patients (or *atm*$^{-/-}$ mice) points to the existence of significant deficits in cell cycle control. We hypothesized that the neurological phenotype in A–T disease could have as one of its roots causes a neuron-specific failure of ATM-dependent cell cycle control. In 2005, Yang and Herrup [23] reported their findings on autopsy specimens from cerebella of individuals diagnosed with A–T. In keeping with our hypothesis, 10–20% of the post-mitotic cerebellar Purkinje and granule cells had detectable levels of PCNA, cyclin A and cyclin B in their nuclei and/or cytoplasm using immunohistochemistry (Fig. 1B). All regions of the A–T brain that have been identified with neuronal cell loss (basal ganglia, striatum), had evidence of ectopic cell cycle proteins in neuronal cell bodies. Little or no staining was found in age-matched control samples, and there was no immunocytochemical signal from regions of the brain that are unaffected in A–T. This evidence points to a situation in which, despite their supposedly post-mitotic status, mature Purkinje cells have re-initiated a lethal cell cycle, suggesting that cell cycle events are clearly associated with neurons at risk for death in A–T. We propose that the pathogenic mechanism that drives the loss of Purkinje and granule cells in A–T is similar in at least some of its core components to other disorders such as Alzheimer's disease. This tight correlation argues that the immunolabeled proteins are closely tied to the neurodegeneration itself and mark a neuron under stress and at high risk for death.

2.4. Ataxia telangiectasia mouse model—atm-deficiency mouse

Mouse models of A–T have been created in different laboratories [81–85] and have been closely studied for hints to the biological basis of the human disease. Engineered mutations in the mouse ATM homolog (*atm*$^{-/-}$) accurately mimic most of the non-CNS features of human A–T [81,82,84–86]; the findings in the nervous system have been less consistent. This is because, in the mouse *atm*$^{-/-}$ CNS, there is no significant loss of cerebellar Purkinje cells. Dislocated and abnormally differentiated Purkinje cells and other neurological defects including behavioral abnormalities and altered electrophysiology, have been seen in these mice [83,85]. Eilam et al. [87,88] reported the reduction of tyrosine hydroxylase-positive nigrostriatal neurons and dopamine transporter in *atm* $^{-/-}$ mice, but biochemical evidence from Mount et al. [89] in the same animal model raises the scepter of additional complexities. We know that ATM responds to DNA damage ([90–93]. We also know that, through its targeting of p53 for phosphorylation, ATM protein plays a role in neuronal death [94]. Further, mutations in NBS1 and hypomorphic mutations of the MRE11 gene are associated with human neurological defect—Nijimegen breakage syndrome and ataxia–telangiectasia-like disorder, respectively [95–97]. Nbs1 and Mre11 associate with Rad50 to form a larger molecular structure known as the MRN complex. This complex is both a target and a direct inducer of the ATM kinase and it plays a role in DNA double strand break processing and checkpoint signaling.

All of this information, however, has been gained though the study of cultured cell lines. Understanding the role of ATM in the context of the post-mitotic neuron of the central nervous system remains a great challenge. In the study by Yang and Herrup [23] , the authors showed elevated cell cycle proteins (cyclin A and PCNA) in both mouse Purkinje cells (Fig. 1D) and neurons of the striatum, just as they had found in the human brain. They confirmed that the immunostaining represented a coordinated cell division program by using the patterns of FISH labeling to demonstrate that significant DNA replication had occurred in these 'post-mitotic' Purkinje cells (Fig. 1F). Thus a cell cycle process has begun in these cells and proceeded through most or all of S phase. As in the human A–T situation, this cell cycle activity is associated with the degenerative process of neurons. The observations suggest that failure of cell cycle regulation is a unifying feature of the disease process in all affected A–T tissues, explaining not only the radiation sensitivity and cancer predisposition in A–T, but also the neurodegeneration. The *atm* mouse model shows that loss of cell cycle control occurs in the *atm* mice at levels comparable to those found in human A–T. The FISH data reveal that a true cell cycle has been initiated since significant genomic DNA replication occurs in the mouse *atm*$^{-/-}$ Purkinje cells. The RB-deficient mouse, the granule cell death in *lurcher* and *staggerer*, and the results of neuronal tissue culture experiments all show that initiating a cell-cycle is a lethal event; for a neuron, loss of cell cycle control results in cell death, not tumorigenesis. Abortive cell cycle re-entry is tightly correlated with the degeneration of Purkinje cells in individuals diagnosed with ataxia–telangiectasia and its mouse model. This suggests that mature Purkinje cells can re-initiate a lethal cell cycle in both mouse and man and further suggests that the loss of Purkinje and granule cells in A–T may share a common pathogenic mechanism with other neurodegenerative disorders.

2.5. Abnormal cell cycle and other neurodegenerative diseases

Activation of cell cycle machinery has also observed in vulnerable regions in several other neurodegenerative disorders. Postmortem brain tissue studies of stroke or ischemia have revealed evidence for the reexpression of cell cycle proteins such as PCNA, cyclin D and GADD34 [98,99]. The appearance of these cell cycle proteins at very early times after middle cerebral artery occlusion (MCAO) strongly suggested that the initiation of a cell cycle is an early event in the process leading to cell death. Cell cycle markers have also been detected in the brain of patients diagnosed with Parkinson disease [100] and amyotrophic lateral sclerosis [18] . Ectopically expressed cell cycle proteins have also been reported in frontal–temporal dementia, in Niemann–Pick disease, Pick's disease, and in progressive supranuclear palsy [101]. Evidence of cell cycle related cell death has also been observed in the experimental in vivo model of Parkinson's disease [102] , as well as recent reported in the mouse models of both amyotrophic lateral sclerosis [18,103] and the related SOD-1 transgenic mouse model [104].

3. Cell cycle events: do they protect or kill neurons?

The *atm* mouse highlights the same problem that we first ran across with the AD mouse models: cell cycle positive neurons (with elevated expression of cell cycle proteins and supernumerary copies of several DNA loci) can and do survive for months. Neither the APP transgenic mouse models of AD nor the *atm*-deficient mouse suffer any significant loss of neuronal cell bodies even many months after these cells have initiated a cell cycle and replicated their DNA. Yet in human AD or AT disease the loss of neurons is profound (and the neurological phenotype is proportionately more severe). The reason for the discrepancy between the human and mouse neurodegenerative phenotype is unclear. How can these findings are synthesized into a single view of the life and death of the neuron.

We know that the appearance of aberrant neuronal cell cycle is closely related to the findings of neuronal degeneration in human brain. In the mouse, cell cycle re-entrance consistently occurs in three different APP mouse models of AD and in *atm*-deficient mice. In the mouse, the cell cycle events are found early in the disease process, before significant pathologic findings (months before the plaques in APP transgenics; during the Purkinje cell dendritic development is complete in A–T). Yet studies of both AD and *atm*$^{-/-}$ mouse models showed that neurons do not die even after reentry into the cell cycle. The discordance might suggest that a somewhat different perspective of the human situation might be called for.

There would seem to be two basic interpretations of these observations. The first is that the cell cycle events themselves are necessary, but not sufficient to induce the death of adult CNS neurons. Perhaps, just as in cancer, a 'second hit' is needed [105]. One such triggering event could be hypoxia–ischemia, or reactive oxidative stress, as three recent papers implicate cell cycle events in the neuronal death following hypoxia–ischemia in rodents [106,107]. In addition, recent studies in transgenic mice producing wild-type human tau, in the absence of endogenous mouse tau, have documented the formation of neurofibrillary tangles and neuronal cell death associated with altered cell cycle events [108]. The suggestion is that different tau isoforms or tau expression patterns are potential triggering events in a complex web of cytological interactions. In keeping with this proposal, the transgenic lines with altered tau homeostasis have more reliably been associated with neuronal cell death than other transgenic models.

The cell cycle events are correlated with neuronal cell stress in the adult CNS, but correlation is not proof of causality. Another intriguing possibility is that, for unknown reasons, the CCEs we observe are actually protective responses of a neuron under stress, and that much as in certain developmental situations [109] the neuron benefits from having multiple copies of each allele. The end result of a neuronal cell cycle is the death of the cells, but the long times required between initiation of the cycle and death suggests that *in vivo* there are other factors that must be involved in the death process.

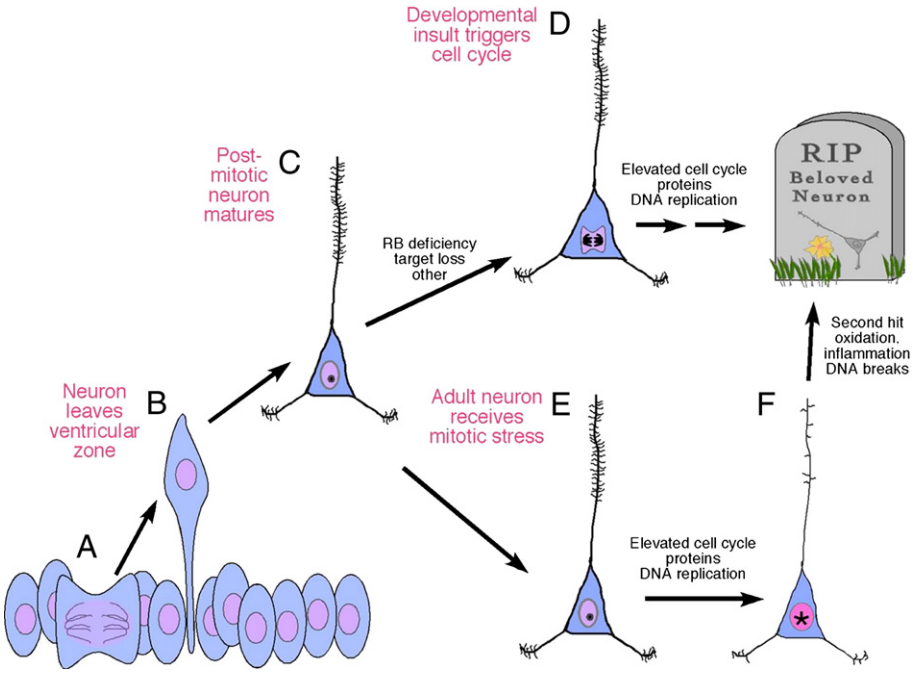

Fig. 2. Cell cycle associated cell death in neuronal cells. (A) During the neurogenic period cells divide rapidly in the region of the ventricular zone. (B) After leaving ventricular zone, neurons become permanently post-mitotic and begin their migration to their final adult location. (C) During and after migration is complete the neurons completes its morphological and biochemical differentiation program. (D) Before they have reached a certain state, any interruption of cell cycle control results in the neuron re-entering a cell cycle followed by death within hours. (E) In a mature neuron, any mitotic stress or stimulus that induces neuron reenter into a cell cycle will lead to a state which is permissive, but not sufficient to lead to neuronal death. (F) We hypothesize that a second "hit" (oxidative, inflammation and DNA breaks) is needed to push the process of cell death in the adult neuron.

It is noteworthy that in a wide variety of different neurodegenerative conditions, these events are reliable and early markers of neuronal distress. Cell cycle is a permissive event for vulnerable neuronal cells, but the final death of the neuron likely requires a second hit. Several possible stimuli such as chronic inflammation, hypoxia and/or DNA damage, oxidative stress may serve this function and force neuronal cells that are already under cell cycle stress to commit to death *in vivo* and in experimental *in vitro* systems. This second event is an environmental insult of either chemical or physical nature to further affect on stressed cycling cell (Fig. 2: the scheme of hypothesis).

If we could learn more details of the underlying biology of the forward reaction we will learn enough about the 'kinetics' of the process that we can provide conditions that favor the reverse reaction in both mouse and human. It would offer prevention strategies of potential clinical importance.

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